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ARTICLE

Reproductive Physiology of *Aedes* (*Aedimorphus*) *vexans* (Diptera: Culicidae) in Relation to Flight Potential

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ABSTRACT Total protein, lipid, and glycogen of *Aedes vexans* (Meigen) were related linearly to body size at eclosion. Starvation after emergence led to the determination of minimal irreducible amounts of protein, lipid, and glycogen and the availability of the teneral reserves, whereas access to sucrose revealed the potential for reserve synthesis. Glycogenesis and lipogenesis increased reserves ≈10-fold the teneral value within 1 and 2 wk after emergence, respectively. Carbohydrate feeding was an essential behavior before blood feeding and oogenesis commenced. Female flight was tested on a flight mill. Maximal flights of 10–17 km in a single night occurred at 2 wk posteclosion and paralleled maximal reserve syntheses. Comparisons of our laboratory data to host-seeking mosquitoes in the field confirmed our data. The vast majority of maternal lipid was transferred to the yolk when a blood meal was taken, but only a quarter of the blood protein was recovered from mature ovaries. Maternal glycogen was used mainly for flight. Fecundity varied between 20 and 120 eggs per female and was determined largely by body size and blood meal volume. At 27°C, maximal egg numbers were produced, but at 22 and 17°C the caloric yolk content was greater. Females from the southern United States were smaller than females from northern areas. However, southern females had similar fecundity as northern females, and their flight performances were similar. Differences in the reproductive physiology between this species and *Ae. aegypti* were discussed.

KEY WORDS *Aedes vexans*, physiology, flight, size, reproductive potential, reserves

THE FLOODWATER MOSQUITO, *Aedes vexans* (Meigen), is an important pest species during spring and early summer throughout the holarctic region. Given suitable flooding regimes, large populations emerge and persistently bite humans in large numbers, impacting tourist or recreational activities resulting in substantial economic loss (N. Becker, personal communication). Furthermore, *Ae. vexans* is the reputed vector of Tahyna virus in Central Europe (Pilaski and Mackenstein 1985, Pilaski 1987).

Aedes vexans has been reported to be a migratory species, capable of long flights from larval habitat (Horsfall et al. 1973). Exodus flights of over 100 km begin at dusk and continue throughout the evening, whereas appetential flights of <15 km have been reported (Horsfall et al. 1973).

Large populations of this species are maintained by an extended diapause of up to several years within the egg-shell at the oviposition sites (Horsfall et al. 1973). Therefore, survival of the species also depends on its flight performances between breeding sites and blood sources.

The reproductive physiology of *Ae. vexans* is poorly known. Feeding on nectar was observed for males and females (Horsfall et al. 1973). Vargo and Foster (1984) found that nectar feeding was most frequent among nullipars, but continued throughout imaginal life. Autogeny appears to be absent in this species. In addition to humans, horses, cows and dogs are recognized as blood meal sources, as well as birds (Horsfall et al. 1973). Preferred feeding times are near sunset, but also occur during daytime when increased humidity prevails (H.B., unpublished data). Blood-feeding appears to be preceded by copulation (Horsfall et al. 1973). The average human annoyance time following emergence lasts about 10 d. Blood meal volumes were reported to be between 2 and 4.7 mg per female. The number of eggs matured by wild-caught females varied from 108 to 182, with an average of 132 (Horsfall et al. 1973).

Breeland and Pickard (1964) described up to eight blood meals per female lifetime, resulting in a total of 1–12 egg batches, with a mean of 54.9 ± 14.1 ($N = 30$) eggs per batch. Oviposition may require preoviposition periods of 5–25 d, starting 1–2 d after voiding the hematin (Breeland and Pickard 1964). Longevity was between 3 wk and 6 wk, but in the laboratory was 42 d at 25°C and 82 d at 13°C when fed honey (Horsfall et al. 1973). Costello and Brust (1971) observed maximal survival times of ≈70–80 d for females and up to 60 d for males, depending on feeding on honey, temperature, and relative humidity. Under starvation maximal survival was 9 d at 21°C, but reached 42 d at 13°C for females (Costello and Brust 1971).

More recently, Van Handel and Day (1988) measured the lipid and glycogen reserves for resting field females. The caloric lipid content exceeded 2–3 times

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the reserve glycogen, and varied in relation to body size. They also pointed out the difference between the constant hemolymph trehalose of 0.02–0.04 cal per female and the highly variable crop sugar contents.

In view of the scant knowledge of the physiology and public interest in this noxious species, we report herein our studies of the reproductive physiology of *Ae. vexans*, including teneral status and body size, reserve synthesis, survival conditions, fecundity, and flight potential of females of increasing age. Our data revealed reproductive strategies remarkably different from *Aedes aegypti* (L.) or *Anopheles* species (Briegel 1990a, 1990b).

Materials and Methods

Eggs of *Aedes (Aedimorphus) vexans* (Meigen) were collected at Kühkopf Island in the Rhine River (Germany: 8° 27′ 56″ E, 49° 48′ 58″ N; strain KK) by R.K. and a colony was established in 1975. In some experiments, we compared KK to strains collected in 1995 in the Sonoran desert in (California: 114° 29′ 40″ W, 30° 49′ 30″ N; strain CAZ), and from Winnipeg, Canada, collected in 1993 (97° 9′ 0″ W, 49° 53′ 0″ N; strain WPG). All colonies are maintained at the University of Mainz. In addition, we also examined females collected by H.B. at Wienacht (9° 3′ 4″ E, 47° 27′ 54″ N) and pupae from Maschwanden (8° 25′ 0″ E, 47° 14′ 6″ N), both rural villages in Switzerland. However, most of the material used for this study was generations 144 and 145 of strain KK, hatched from eggs sent from Mainz to Zurich. Larvae were reared in polystyrol pans (25 by 18 by 5 cm) at a density of 300–500 larvae per pan with 400 ml of distilled water, and fed Tetramin daily according to a schedule adopted from Timmermann and Briegel (1993). Imagoes of large and small body sizes were obtained by manipulating the larval food supply. The larval rearing and all experiments were performed at 22 ± 1°C under natural daylight. Further details about colonization are reported separately (R.K., unpublished data). Blood meals were taken from a human arm. Oviposition was never complete in our laboratory, and therefore females were dissected 60–72 h post blood meal to examine their ovaries, count the mature oocytes, and determine the caloric composition of yolk. To test fecundity, some experiments were carried out with females kept at 17 or 27°C after blood feeding. Mating status was not considered. Wing lengths were routinely measured (mm) and their cubic value (mm^3), henceforth, is defined as body size (Briegel 1990a). Survivorship curves were established by following cohorts of 50 newly eclosed females held in round cages (diameter 12 cm, height 14 cm).

Flight mills were described previously (Briegel et al. 2001). The revolutions were counted by computer at 30-s intervals. The total distance flown was determined with each spike being the signal for a distance flown per time unit (m/s). All flight protocols were analyzed for temporal flight patterns and pauses on print-outs. For each run, one female was glued to the tip of an arm with a tiny drop of wax on its scutum. On the opposite arm a female of identical body size was mounted with its wings glued together to provide a passive flight control. Identical females were kept individually in narrow tubes, limiting their movement and serving as an additional "non-flown" control. At the end of the flight trial, all females were fixed for biochemical analyses of their reserves. For comparison, females from the same cohort also were fixed at the outset of each experiment and used as preflight controls. Flight trials routinely started in the evening and lasted overnight for 18–20 h to minimize disturbance by human activity in the laboratory. Females dying before 18 h were discarded.

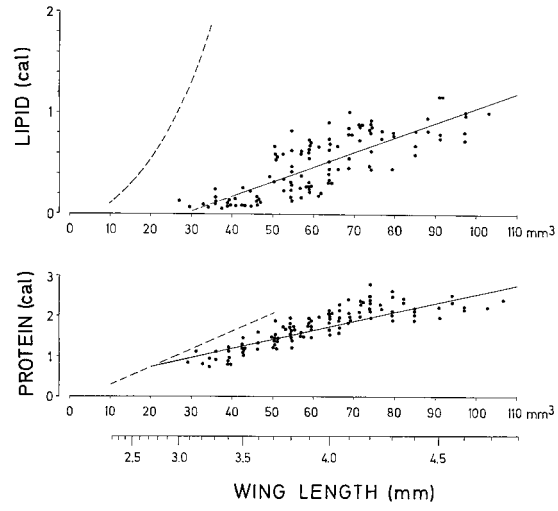

Fig. 1. Caloric protein and lipid content at emergence of female *Aedes vexans* in relation to body size (WL^3) expressed as the cube of wing length. For comparison, the corresponding regressions for *Ae. aegypti* have been added as a dashed line (data from Briegel 1990a).

For biochemical analyses of lipid and glycogen we used the methods of Van Handel and Day (1988) as described earlier (Timmermann and Briegel 1993). For protein determinations samples were digested by a Kjeldahl procedure with subsequent Nesslerization (Minari and Zilversmit 1963). All data are expressed as calories per female; sometimes the caloric data were normalized for body size, leading to so-called size-specific caloric contents (SSCC-values; Timmermann and Briegel 1993). Linear regressions were computed on a calculator (HP-97); *t*-test was applied for significant differences of means.

Results

Prebloodmeal History. Manipulation of larval food and density produced males and females of variable body sizes. The total protein, lipid, and glycogen content of newly emerged imagoes was measured, and the respective caloric values plotted as a function of body size, expressed as the cubic value of the wing length (Fig. 1). Linear regressions followed for females were as follows: protein: $Y = 0.022X+0.312$ ($N = 107$, $r^2 =$

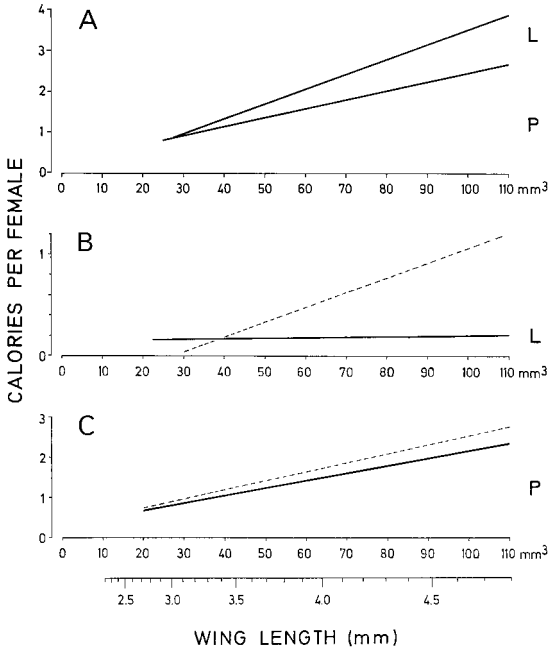

Fig. 2. Female body sizes in relation to protein (P) and lipid (L) contents in *Ae. vexans.* (A) Cumulative presentation of teneral protein plus lipid values (regressions as in Fig. 1). (B) Minimal irreducible amounts of lipids, i.e., in females starved to death; the regression formulas for lipid was Y = 0.0005X+0.136 ($N = 61$, $r^2 = 0.018$, $t = 1.03$, $P > 0.3$). (C) Minimal irreducible amounts of protein: Y = 0.016X+0.319 ($N = 40$, $r^2 = 0.640$, $t = 8.23$, $P < 0.001$). Dashed lines in B and C are the teneral regressions to indicate the extent of lipid and protein disappearance during starvation.

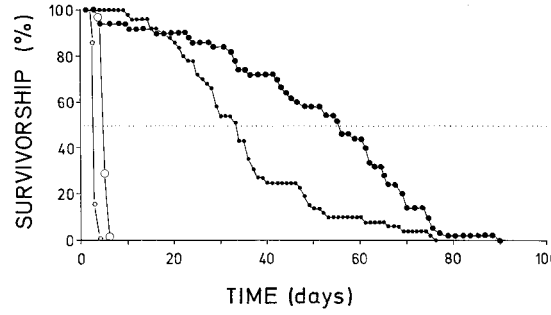

Fig. 3. Survivorship curves for large and small females *Ae. vexans* fed sugar 10% (●) or only water (○). Large symbols for cohorts of large females, small symbols for small ones. The dotted line marks the 50% survival time.

0.582, $t = 12.10$, $P < 0.001$), lipid: Y = 0.015X-0.418 ($N = 109$, $r^2 = 0.645$, $t = 13.92$, $P < 0.001$), glycogen: Y = 0.002X-0.033 ($N = 109$, $r^2 = 0.368$, $t = 7.89$, $P < 0.001$). The absolute ranges of the individual values were 0.5–2.5 cal protein, up to 1.2 cal lipids and below 0.2 cal for glycogen. In Fig. 2A the protein plus lipid regressions were compiled to illustrate the caloric composition at emergence that varied from 1–4 cal per female, depending on body size.

For males the following regressions were obtained: protein: Y = 0.021X+0.171 ($N = 62$, $r^2 = 0.828$, $t = 16.97$, $P < 0.001$), lipid: Y = 0.013X-0.148 ($N = 80$, $r^2 = 0.526$, $t = 9.23$, $P < 0.001$), glycogen: Y = 0.002X-0.005 ($N = 80$, $r^2 = 0.286$, $t = 5.59$, $P < 0.001$)

When mosquitoes were subjected to starvation by providing water from eclosion until death, the minimal irreducible amounts of protein and lipids required for survival were revealed (Fig. 2 B and C). The results of this experiment indicate the extent of reserves that can be mobilized without dietary input. The minimal female lipid amount was extremely low and showed no significant correlation with body size (Fig. 2B), whereas for glycogen an equally low but significant linearity was observed: Y = 0.0005X-0.003 ($N = 61$, $r^2 = 0.401$, $t = 7.62$, $P < 0.01$). Protein also showed a significant linear regression, revealing a reduction of only 15% of the teneral values (Fig. 2C). In contrast, large females mobilized >84% of the lipid (Fig. 2B) and 50–70% of the teneral glycogen.

Female and male cohorts required 4–6 d until death due to starvation (Fig. 3). Based on the caloric differences between minimal irreducible amounts and teneral values, an average daily rate of glycogen and lipid mobilization for survival was calculated as follows: 0.01–0.02 cal of glycogen/day/female and 0.08–0.13 cal of lipid/day/female. In contrast, when offered 10% sucrose ad libitum, small females (3.85 ± 0.30 mm) survived for 76 d and large females (4.20 ± 0.20 mm) for 90 d; the median survival times were 33 d and 55 d, respectively (Fig. 3).

Females provided with sugar ad libitum were analyzed for total lipid and glycogen at intervals from emergence until 24 d. They clearly synthesized lipid and glycogen. Although glycogen rarely exceeded 1 cal/female, maximal lipids were = 7 cal/female. When standardized for size and related to the teneral value (arbitrarily set 1.0), the relative gain of reserves was determined. Fig. 4 shows glycogen and lipid data plotted for the same two size-classes at 4-d intervals after emergence. Lipids and glycogen increased roughly 10 times each per mosquito during the first week of imaginal life and reached over 20 times the teneral values within the second week. Glycogen curves generally had a higher rate of synthesis than for lipids. This pronounced glycogenesis and lipogenesis may allow survivorship to be extended from day 20 to 80 (Fig. 3).

The reserve status of host-seeking females was determined for females collected in the field when landing on a human bait but before piercing the skin. Their protein content was similar to teneral females, but their lipid and glycogen contents were increased substantially (Figs. 4 and 5). Lipid values were between 1.2 and 5.0 cal/female and the absolute glycogen values of the same females ranged from 0.1 to 0.6 cal/female. When adjusted for size, values represented a 3- to 8-fold increase in lipids and a 2- to 7-fold increase over glycogen at emergence. This agreed with the sugar-feeding data from the laboratory, observed during the first week of life (Fig. 4). These females had glucose values ranging from near zero to 1 calorie.

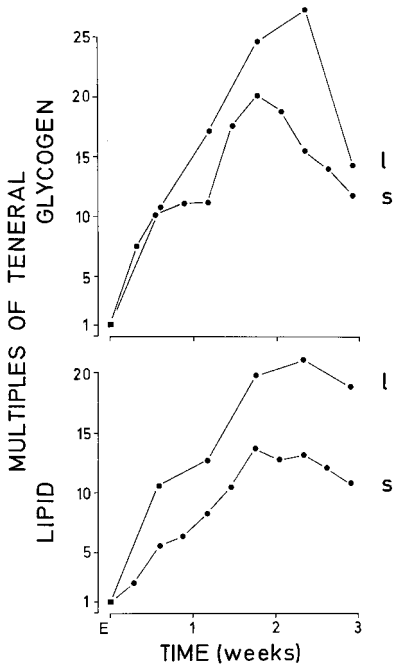

Fig. 4. Relative reserve synthesis of female *Ae. vexans* with permanent access to 10% sucrose for 3 wk after emergence (E). Caloric values have been standardized for size, and then expressed in multiples of the teneral value (1.0, square symbol). Both measurements are from the same individuals of large (l) or small (s) body sizes which belong to the same cohorts as in Fig. 3.

These measurements represent crop contents of sugar, ingested before host-seeking.

Blood Meal and Fecundity. Blood meals were offered to starved females on a human arm at intervals after emergence. Females initially probed on day 2, but feeding was observed only after day 3 and in <10%

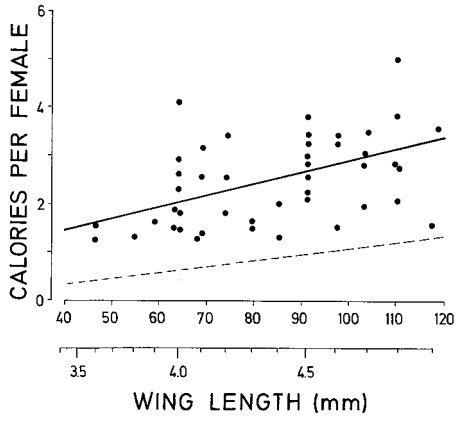

Fig. 5. Regression of lipid content in field host-seeking *Ae. vexans* as a function of size: Y = 0.024X+0.516 (N = 45, r^2 = 0.284, t = 4.13, P < 0.001). The dotted line is the regression of teneral females colonized in the laboratory (from Fig. 1).

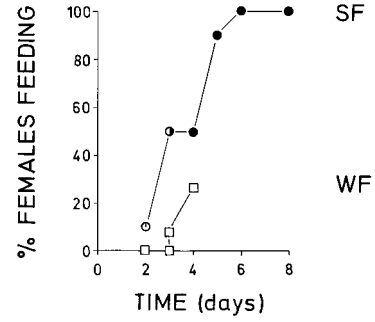

Fig. 6. Feeding response of female *Ae. vexans* on a human host when maintained on water (WF open squares) from eclosion, or offered sucrose 10% (SF). Sugar-fed females gradually became oogenic, indicated by the extent of black segments in the respective circles, whereas water-fed starving females never entered oogenesis.

of the females (Fig. 6). At day 4, 25% of the water-fed females ingested blood, but oogenesis failed. By day 5, females were either dead or already too weak to ingest blood.

In contrast, the sugar-fed females took their first blood meal on day 2, and 10% of the females matured a few oocytes (Fig. 6). When blood was ingested on day 3, half of the females fed and 50% of those matured eggs. After day 4, all with blood initiated oogenesis, but only after day 6 did all females ingest blood and complete oogenesis. Female *Ae. vexans* therefore require about one week of sugar feeding to attain full competence for blood feeding and completion of oogenesis.

Some host-seeking females from the field were allowed to ingest a full blood meal on the forearm. There was a considerable increase in protein. Females contained between 4.5 and 9.5 cal, which was 3 to 4 times the teneral values when standardized for size. Based on the blood protein titer of H.B., data correspond to blood meals of 3 μl in small females and up to 8 μl in large females. There was broad variability in these field data, perhaps caused by variation in the degree of previous crop distention with sugars.

Oogenesis and fecundity were analyzed in the laboratory. Blood meals were ingested from a human host, after which the females were maintained at 17, 22, or 27°C. Three to 5 d later their ovaries were excised and the mature oocytes counted and fixed to determine the caloric yolk contents. Fecundity was always related linearly with body size: Y = 1.21X+4.76 (N = 71, r^2 = 0.464, t = 7.72, P < 0.001), with a range of 20–120 eggs per female. Surprisingly, when kept at 27°C after blood feeding, fecundity was reduced by 25%, but still was related linearly to body size: Y = 1.00X+0.53 (N = 47, r^2 = 0.197, t = 3.32, P < 0.01).

There were significant linear correlations between oocyte number (X) and ovarian protein (Y = 0.011X+0.011; N = 55, r^2 = 0.904, t = 22.41, P < 0.001) or ovarian lipid content (Y = 0.015X+0.382, N = 54, r^2 = 0.503, t = 7.25 p ≪ 0.001), regardless of temperature. Yolk content averaged 11.4 mcal protein per

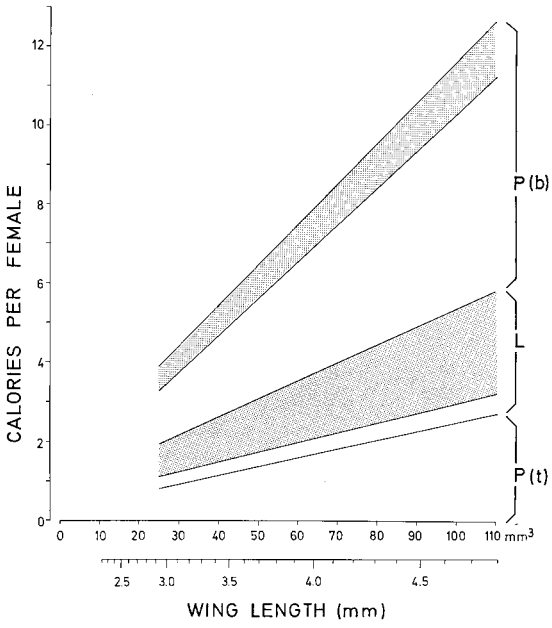

Fig. 7. Compilation of protein and lipid utilization for oogenesis in *Ae. vexans*, based on the preceeding regression lines. P(t) represents the teneral protein content; L is the total lipid content per female at the time of blood meal, composed of the teneral values plus sugar-derived lipogenesis; P(b) indicates the additional protein obtained through blood meal. The gray areas denote the segments of protein or lipid recovered from mature ovaries.

oocyte and 20.3 mcal of lipid per oocyte, independent of holding temperature.

The utilization of blood meal protein for synthesis of yolk protein (Y) was size-dependent: $Y = 0.009X+0.403$ ($N = 32$, $r^2 = 0.150$, $t = 2.30$, $P < 0.05$). Similarly, utilization of maternal lipid for synthesis of yolk lipid (Y) was also size-dependent: $Y = 0.021X+0.294$ ($N = 37$, $r^2 = 0.268$, $t = 3.58$, $P < 0.001$). These regressions were valid for females kept at 17° or 22°C after blood feeding. When kept at 27°C, females produced fewer eggs and the yolk content was 1.403 ± 0.440 lipid cal/female ($N = 18$), and 0.806 ± 0.284 protein cal/female ($N = 23$); 10% less lipid and 16% less protein than in females held at cooler temperatures. To illustrate the distributions between maternal and ovarian protein and lipid, we present a cumulative diagram in Fig. 7, including all female body sizes. Up to 80% of the pre-blood meal maternal lipid were transferred to the ovaries. With protein there was a different situation; ≈75% of the blood meal protein was not used for oogenesis but its metabolic and catabolic fate was not determined (Fig. 7).

We compared these fecundity data with two North American strains; WPG and CAZ (Tab. 3). Body sizes were much smaller in the southern strain (CAZ: wing length 3.4 mm) than in the northern strain (WPG: wing length 4.2 mm), whereas the European strain was in between (KK: wing length 4.05 mm), despite identical larval rearing conditions and sufficient amounts of food.

Mean blood consumption increased with body size, but when expressed as a size-specific caloric protein (SSCC-values in Table 1), it was higher for CAZ than for KK. In agreement with the different protein meals, fecundity was significantly higher in CAZ compared with WPG ($t = 2.53$, $P < 0.02$). The protein and lipid content per oocyte in milli-calories also are shown in Table 1. KK females deposited 59% more lipid and 40% more protein into their oocytes than the southern CAZ strain, indicating that CAZ females produced more eggs with smaller caloric content. Furthermore, there was a fairly constant proportion of 2:3 between protein and lipid yolk in all three strains. These trends may relate to longer diapause periods required in the northern strains, and are supported by the content of caloric yolk lipid per egg.

Flight Activity and Metabolism. The flight performance of *Ae. vexans* under controlled experimental conditions using water-fed and sugar-fed females of increasing age were tested on the flight mill for 18–20 h (Fig. 8). Each flight protocol of 170 sugar-fed females was analyzed for the total distance flown and for the temporal flight pattern. If we rejected flights <1,000 m per night, a total of 132 females remained, which were divided in two groups: average flights (82 females between 1 and 5 km per trial), and strong flights (50 females >5 km). With access to sucrose, the flight performance increased every day (Fig. 8). The development of the strong flights is demonstrated in Fig. 9, where age-related distributions of the two flight categories are shown, based on the 132 flight protocols. During the first week the majority of flights were average flights. The strong flights appeared gradually, and within the second and third week they exceeded the 50% level; maximal flights were 15–17 km per female per night. In contrast, starved, water-fed females showed an average flight distance of only 510 m at day 1, 470 m

Table 1. Comparison of blood protein input (SSCC-values), fecundity (eggs per female), and the two prominent yolk components (mcal per egg) among females of three different strains with different body sizes (WL^3)

Strain	*N*	Body size	Protein input	Eggs	Yolk	
					Protein	Lipid
CAZ	24	38.83 ± 5.31	0.109 ± 0.02	99 ± 21	8.9 ± 0.6 (12)	11.4 ± 2.0 (11)
WPG	18	73.91 ± 8.05	0.067 ± 0.019	82 ± 17	11.3 ± 0.5 (7)	15.5 ± 1.7 (7)
KK	18	66.67 ± 8.86	0.084 ± 0.018	94 ± 19	12.5 ± 1.0 (9)	18.1 ± 3.0 (9)

Means ± SE, *N* in parentheses.

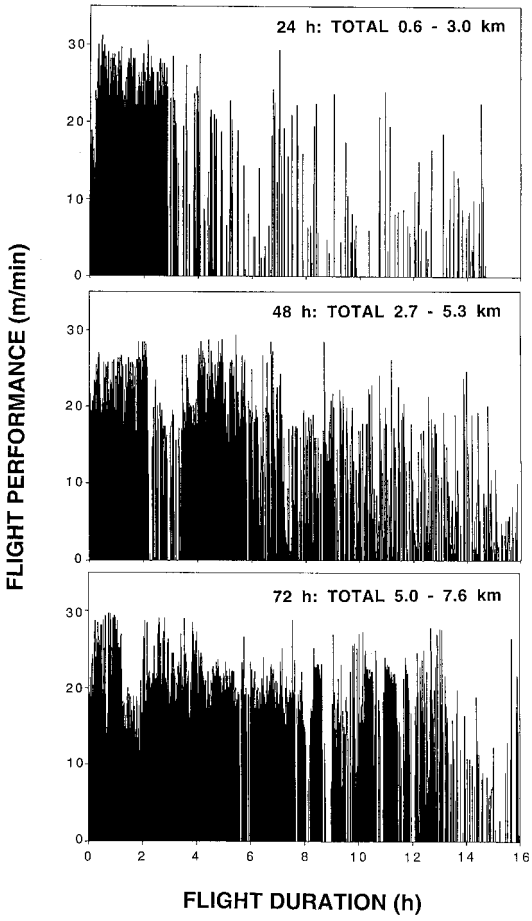

Fig. 8. Flight performances of three females of *Ae. vexans* from day 1 to 3 after eclosion, with access to sucrose before flight. The range of total distance flown by each group is indicated. Each spike represents a flight period, and the solid black segments show periods of continuous flight.

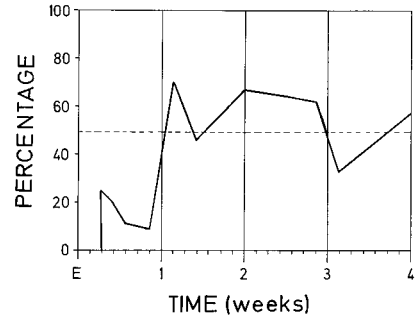

Fig. 9. Development of strong flight activities of *Ae. vexans.* Frequencies of average (above dashed line) and strong fliers (below dashed line) during the first 4 wk of imaginal life with access to 10% sucrose are given as percent of the respective cohorts.

at day 2, and 20 m at day 3 after eclosion, with a maximum of 1.36 km, after which they died.

Males were poor fliers. At day 1, starved males reached a mean distance of 270 ± 192 m (maximum 565 m); this decreased to zero at day 3 when they soon died. With sucrose, mean distance at day 1 was 1.12 ± 0.60 km, with a maximum of 3.15 km at day 2. Afterwards their mean distances remained far below one km until day 8. It appeared that males depended heavily on sugar-feeding early in life.

Flight data were examined for temporal patterns, i.e., screened for continuous, nonstop flight segments. Flight segments <15 min were discarded, whereas flights of 30–60 min were considered average, and flight segments of >1 h were considered strong. With these conditions, 204 females were analyzed. For average fliers, the mean time of continuous flight was 0.71 ± 0.05 h (N = 167; or 45 ± 3 min), whereas for the strong fliers continuous flight lasted 1.9 ± 0.3 h (N = 118). With the exception of one female with 9 h of continuous flight, the 10 best fliers averaged nonstop flights of 6.2 ± 1.1 h. Therefore, roughly a third of the time and of the distances flown may be attributed to continuous flight segments that were spaced by intermittent pauses.

By plotting the total flight distance against total flight time, a significant linear regression was obtained of Y = 0.847X+1.519 (N = 224, r^2 = 0.601, t = 18.24, $P < 0.0001$). Flight speeds were calculated for this regression; maximal values of 1.7 km/h were observed, but the average speed was ≈1 km/h and largely independent of sex, food, or general activity of the mosquito.

Metabolic aspects were studied by analyzing the glycogen and lipid reserves of sugar-fed females of increasing age before and after flight. Controls were females mounted on the opposite arm of the flight mill with their wings glued together. A typical result for females of the KK strain is summarized in Table 2 for strong flights and average flights. During flight glycogen was reduced by 0.24 cal (35%) in females with fixed wings (passive fliers) and by another 0.39 cal (56%) by strong fliers. Therefore, a total of 91% of the preflight glycogen was used (=0.63 cal per female) during strong flight, reaching a mean distance of 7.04 km. In average fliers these caloric values were similar: 94% (0.65 cal per female) with 2.98 km.

Lipid measurements revealed a decrease of 0.25 cal per female (3.7%) during strong flight, and 1.37 cal/female (20.3%) for average fliers (Table 2). Because caloric lipids after strong (6.49) and average flights (5.37) were not significantly different (Table 2), there was no clear trend recognized for higher energy requirements during strong flight activities.

In Table 3 we compare the flight metabolism among the three strains of different geographic origin during their periods of best flight performance. Although there was some variability, ≈0.53 cal of glycogen (91%) and 1.17 cal of lipid (33%) were used, or 0.08 cal of glycogen and 0.18 cal of lipid per kilometer. Despite the different body sizes, glycogen utilization per flight distance was fairly constant, with 0.08 cal/km (an average of 91% of the preflight value). The synchronous utilization of lipids with 0.18 cal/km was about

Table 2. Comparison of caloric glycogen and lipid contents before and after active and passive flights in female *Ae. vexans* (strain KK) fed sucrose ad libitum before the flight experiments

Flight conditions	Glycogen		Lipid		Distance flown, km
	(calories)	%	(calories)	%	
Before flight	0.69 ± 0.18 (7)	100	6.74 ± 2.47 (7)[a]	100	
After passive flight	0.45 ± 0.15 (7)	65	5.73 ± 1.27 (7)	85	
After strong flight	0.06 ± 0.03 (6)	9	6.49 ± 1.85 (6)	96	7.04 ± 1.02[b]
After average flight	0.04 ± 0.03 (6)	6	5.37 ± 1.42 (14)[a]	80	2.98 ± 0.54[b]

Data are based on daily means and chosen for days 8–22, when optimal flight performance was observed (Fig. 9); mean ± SE (*N* in parentheses).

[a] Difference in lipids are not significant ($t = 1.63$, df = 19, $P > 0.1$).

[b] Ranges are 5.14–12.63 km/female for strong fliers and 1.14–4.70 km/female for average fliers.

twice that value, but only 33% of the preflight level. This points toward a segregation between flight-related, constant glycogen utilization and metabolic lipid utilization.

Discussion

The physiology of *Ae. vexans* differs markedly from what is known about *Ae. aegypti* and some *Anopheles* (Briegel 1990a, b). Females emerge with a larger body size (3–4.5 mm versus 2.2–3.7 mm), but carrying much less reserves than *Ae. aegypti* (Briegel 1990a). They are low in protein and lipids, both approximate the range of values for Anophelines. When standardized for size (SSCC-values), these relationships were more prominent: the teneral lipids of female *Ae. vexans* varied between 0.002 and 0.012 cal, close to *Anopheles* with 0.004–0.018 cal, but much less than *Ae. aegypti* with 0.010–0.060 cal (Timmermann and Briegel 1993). This low reserve status also was reflected in a short survivorship of only 3–5 d when starved. With access to carbohydrates, however, survivorship was extended to a maximum of 3 mo in large females. This was accompanied by an enormous synthesis of reserves. With ingestion of sugar, within 1–2 wk glycogen synthesis reached up to 20-fold teneral values and lipogenesis up to 10-fold teneral values. Therefore, the first week after emergence was crucial for imaginal life and sugar-feeding was an essential behavior for this species. Host-seeking was postponed for several days and took almost 1 wk until all females ingested their first and full blood meal and initiated complete oogenesis. Although water-fed, starving females attempted to take blood, many failed and those that fed on blood never initiated oogenesis, often egesting their blood meals overnight. This again is strong evidence for the significance of pre-blood meal carbohydrate feeding to compensate for the insufficient synthesis of larval reserves, especially lipids. This notion also was supported by the prevalence of nulliparous females observed on nectar sources in the field (Vargo and Foster 1984).

The majority of maternal lipid reserves were transferred to the maturing ovaries. Females may only become oogenic when they have acquired sufficient lipid reserves, i.e., ≈1.5 cal per female, corresponding to the amount transferred to oocytes; all teneral females were below this threshold. This may explain the failure of oogenesis in blood-fed, but previously starved females, as observed by many workers. Host-seeking females caught in the field carried equally high lipid reserves. Calculated per single mature oocyte, lipid content was often twice as high as protein content. Because females from northern areas were larger, following Bergman's rule, and deposited more lipid yolk, we believe the caloric dominance of yolk lipids to be of adaptive value for the eggs that are known to enter periods of quiescence of up to 12 yr (R.K., unpublished data). There was a surprisingly low efficiency of the utilization of blood protein for vitellogenesis: only 10–20% of the input. We have not yet quantified the metabolic or catabolic fate of the remaining 80–90% of the blood meal protein. A similar situation was found in *Ae. Ochlerotatus cantans,* which also required a period of at least 2 wk before blood meals were ingested (Renshaw et al. 1995). This interval was characterized by a five-fold increase of lipid reserves over teneral conditions. However, in the laboratory, blood meals were taken earlier when the teneral lipid content had been doubled (Renshaw et al. 1995). It is interesting to note that although *Oc. cantans* is a species with larger body size and greater

Table 3. Comparison of flight metabolism among females (f) of three geographic strains of *Ae. vexans* during vigorous flight activities

Strain	*N*	Size (WL^3)	Flight data		Glycogen utilization			Lipid utilization		
			km	h	cal/f	%	cal/km	cal/f	%	cal/km
KK	8	79.51	7.14	7.3	0.65	92	0.09	0.93	11	0.13
WPG	4	80.99	6.63	6.1	0.58	88	0.09	1.72	43	0.26
CAZ	5	44.49	6.30	5.6	0.35	93	0.06	0.87	44	0.14
Average			6.7	5.6	0.53	91	0.08	1.17	33	0.18

Mean caloric utilization per female, its percentage of preflight values (100%), and the average utilization per 1.0 km flown are combined for days 14–16, the time of best performance.

lipid content (approximately twice the values of *Ae. vexans*), it still required a "maturation time" of at least 2 wk for increasing its lipid reserves five-fold before host-seeking was observed. These authors also included *Oc. punctor* in their studies, a species of similar body size and lipid contents at eclosion as *Ae. vexans.* Yet in *Oc. punctor* host-seeking occurred at day 2, when lipids had hardly doubled since emergence. In that respect the strategy of *Oc. punctor* resembles more that of *Ae. aegypti*, namely faster lipogenesis and earlier blood meals, than in *Ae. vexans* or *Oc. cantans* (Renshaw et al. 1995, Briegel et al. 2001). However, to establish a general rule concerning body size, lipid content, additional reserve acquisition and initiation of a gonotrophic cyle in *Aedes* would be premature at this point.

Aedes vexans has been reported to fly distances of many kilometers (Horsfall et al. 1973). However, field observations often are questionable, because of the possibility for passive transport by wind. With flight mills the maximal distance of active flight observed in our experiments was 17 km. However, this flight was forced, because of the maintained lack of tarsal contact; under natural circumstances they might not have traveled the same distance during one night. In contrast, if additional carbohydrate sources were encountered and ingested, females might be able to engage in even longer flights. The time segments of continuous, nonstop flights of up to 9 h also were unexpected. Short or long pauses between such continuous segments presumably reflected periods of reserve mobilizations.

Comparisons of the reserve status before and after flight trials and between active and passive fliers confirmed that glycogen was the main flight substrate. Nayar and Van Handel (1971) previously traced the fate of labeled ^{14}C-glucose during and after flights and found similar results. Our data from passive flight controls indicate that additional glycogen in similar amounts is used for other purposes during the same periods. Furthermore, we found a simultaneous reduction in total lipid by 10–20% during flight as well as in passive controls. For clarification this result was related to other data in two different ways.

First, because our mosquitoes were starved during the flight experiments, we compared the decrease of lipids to its degradation by females starved to death. The teneral reserves of KK-females were degraded at a rate of 0.13 cal/d/female (0.11 cal lipid plus 0.02 cal glycogen). During flight, however, we observed a total loss of 1.58 cal (0.93 cal lipids plus 0.65 cal glycogen) per female per flight period of 7.3 h (Table 3), which would amount to 5.2 cal/d/female, or 40-times more than under starvation. The fact that during passive flights equal or similar amounts of lipids are used as during active flights might be explained by energy expenditures through covert physical stress, other than flight.

Second, it was informative to compare the energy expenditures with flight data of birds. We have converted values known from the literature for birds to the approximate freshweight of a female *Ae. vexans*, i.e. ≈3 mg. Therefore, passerine birds use an average of 1.5 cal/d/3 mg ($N = 30$) at rest, and ≈10.8 cal/d/3 mg at flight (Louw 1993, Walsberg 1983), a seven-fold increase. As derived from Table 3 and extrapolated to 24 h, females of all three strains of *Ae. vexans* spend an average total of 6.5 cal/d/3 mg. This is well within the range of a flying hummingbird with 4.2 cal/d/3 mg (Walsberg 1983). The energy requirement for heavy duty labor in humans is 0.67 cal/d/3 mg, rendering hummingbird and mosquito flight 6–10 times more expensive.

In conclusion, glycogen in *Ae. vexans* and other mosquitoes is the principal flight substrate, as shown by Nayar and Van Handel (1971). In addition, mosquitoes appear to use lipids at equal rates during passive life and active flight, independent of the actual flight distance, supposedly for metabolic maintenance, as indicated by Van Handel (1965). We therefore suggest that the flying mosquito consists of two different metabolic compartments. The flight muscles in the thorax run on glycogen for several hours before additional mobilization is required. Because the bulk of glycogen is stored in the abdomen, periods for its mobilization and transport are necessary, interrupting flight activities for short or long periods. The other compartment of the organism—in a metabolic sense—appears to be uncoupled from the flight muscle system, running primarily on lipid oxidation, whether flying or not. But this secondary physiological compartment is related strongly to reproduction: host-seeking and the potential for strong flights are manifested only when substantial reserves have been accumulated during the first 2 wk after metamorphosis of *Ae. vexans* and *Oc. cantans.*

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